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LASSA FEVER IMMUNE PLASMA

ANNUAL SUMMARY REPORT

John D. Frame, M.D.

July 31, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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630 W. 168th Street
New York, N.Y. 10032

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18. Immunotherapy
Liberia
19. so that determination of the incidence of LF among febrile patients in these hospitals is not feasible.

Passive immunotherapy with Lassa Fever Immune Plasma (LFIP) was administered to six patients at CLH; all survived. Two were demonstrated to have had LF by virus isolation; in the absence of complete serological testing the diagnosis of other patients is not yet certain. At PH, LFIP was administered to nine patients, two of whom were known to have LF by the isolation of LV; both recovered. In three patients virus isolation has not yet been attempted; one died of fulminating clinical LF. Again, in the absence of complete serological testing the diagnostic status of the others is not clear.

Equipment and reagents for the use of the enzyme linked immunosorbent assay (ELISA) for LF are now at hand in Liberia. The use of ELISA during the coming year will make possible the more accurate diagnosis of LF and the more economical use of LFIP.

Summary

During the second year of the investigation of Lassa fever (LF) in Liberia under contract DAMD17-85-C-5189 the Field Investigator, Mr. J. E. Valley-Ogunro, spent five months at USAMRIID receiving instruction in serological and virological techniques important in LF research. Though not all the procedures will have immediate applicability in Liberia, this education prepares him for a greater role in the conduct and management of the program there.

In part because of his absence from Liberia during this time and in part because of equipment failure at both Curran Lutheran Hospital and Phebe Hospital, our field sites, only 153 units of Lassa Fever Immune Plasma (LFIP) were collected this year; 83 have been designated for the use of USAMRIID.

Lassa virus was isolated at USAMRIID from 27 patients. Of these, 14 isolations were from patients seen the previous year but tested only recently.) Again, as the result of Mr. Valley-Ogunro's absence from Liberia, serological testing has lagged somewhat, and not all specimens have been titered to their end points. Thus, the incidence of LF diagnosed virologically and serologically cannot be calculated at present.

Passive immunotherapy with LFIP was reported in 15 cases. Of these, four were instances of definite LF and one of possible LF. Two recipients died, both pregnant women who had received LFIP after the 10th day of illness.

Consequent upon Mr. Valley-Ogunro's instruction at USAMRIID we expect to begin shortly the use of the enzyme-linked immunosorbent-assay (ELISA) in the diagnosis of LF; with this laboratory assistance it should be possible to diagnose and treat all and only LF patients, with the more rational use of the scarce LFIP resource, and the greater likelihood of determining the treatment's efficacy.

1. *Diagnostic Test Form, ELISA: Diagnosis Medicine (ext)*

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FOREWORD

**For the protection of human subjects the investigators have adhered
to policies of applicable Federal Law 45CFR46.**

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Introduction

The study of Lassa fever (LF) in Liberia continued during this second year of the current investigation. The major activities included further education of the Field Investigator, Mr. J.E. Valley-Ogunro, in diagnostic techniques which will be used in therapeutic investigations, continued studies of LF in Liberian hospitals, and the collection of plasma from convalescents from LF.

The history of LF in Liberia has been summarized in the previous Annual Summary Report, July 31, 1986 (1). The 1972 outbreak of LF among patients and staff at the Curran Lutheran Hospital (CLH) in Zorzor, Lofa County (2) was followed by the demonstration of the high prevalence of antibodies to Lassa virus among staff members (3,4), an incidence of LF of about 15-20% among patients treated in the hospital for fever (5), and a high prevalence of LF in surrounding villages (6). Curran Lutheran Hospital became the first field station for research into LF in Liberia; subsequently studies were started in Phebe Hospital, Bong County. Steps are being taken to ensure adequate logistic support for work at a third field site in Kolahun, in Lofa County.

Lassa fever research in Liberia is performed under the guidance and with the virological support of the United States Army Medical Research Institute for Infectious Diseases (USAMRIID) in Fort Detrick, Frederick, Maryland. USAMRIID supplies the antigenic materials for testing by the indirect fluorescent antibody (IFA) technique. Patient sera are sent to USAMRIID for virus isolation, and for determination of neutralizing antibodies (NA). It has been found in this institution that the efficacy of Lassa Fever Immune Plasma (LFIP), used in the treatment of LF patients, depends upon the titer of NA, as measured by the Log Neutralization Index (LNI) (7).

Because of the USAMRIID finding that titers of NA appropriate for the use of LFIP in treatment do not develop until several months after the onset of infection (7), convalescents are approached for plasma donation 6 months or more after their recovery from LF. At this time a relatively high proportion of donors are found to have titers of NA as defined by the LNI high enough to be considered therapeutic.

Therapeutic trials of LFIP have not been conclusive, though there has appeared to be a beneficial effect in some patients in whom plasma infusion was followed by sudden improvement in the clinical status, an outcome noted as well in some other clinical studies of LFIP (8,9).

In addition to the limited supply of plasma, two other factors confound attempts to investigate the efficacy of LFIP. In Liberia LF has a relatively low mortality of about 5% among non-pregnant adults, so that a very large number of patients would have to be assembled in order to demonstrate the value of treatment as compared with no treatment, if survival of the patients were to be the criterion of

success. The clinical course of LF in Liberia is too variable to permit clear-cut determination of efficacy from the course of illness alone. The other factor is that the laboratory diagnosis of LF is made too late to permit treatment of all and only LF cases. Serological diagnosis is only possible by comparison of titers of specimens taken over the course of several days, and virus isolation requires a containment laboratory such as that at USAMRIID. Some cases are missed by clinical diagnosis alone, and other fevers, not yet clearly defined, often lead to immunotherapy of patients later found not to have LF.

We propose that changes in the levels of LV viremia during the course of treatment become the measure of therapeutic success. We expect that ultrafreezers installed in the hospitals where treatment will be undertaken will permit the maintenance of viral activity to a degree sufficient to permit the comparison of virus titers before and after treatment. The issue of rapid diagnosis will likely be solved by the use of an enzyme-linked immunosorbent assay (ELISA) (10) to permit laboratory diagnosis within hours of a patient's admission to the hospital.

We also plan a trial of another therapeutic modality, ribavirin, an antiviral agent which has been found successful in the treatment of LV infections in monkeys (11) and in humans (12). Inauguration of a trial to compare the use of ribavirin and LFIP, or possibly, Lassa Fever Immune Globulin (LFIG) has been delayed until the use of the ELISA test to monitor treatment is in place.

Activities

In early October, 1986 Mr. J. E. Valley-Ogunro, Field Investigator and Resident Head of the Lassa Fever Control Project of the Liberian Institute for Biomedical Research (LIBR), began a five-month stint at USAMRIID. During this period he was instructed in the techniques of virus isolation, preparation of antigens for the indirect fluorescent antibody (IFA) and the ELISA tests, the use of reagents containing several viral antigens to test for the various African hemorrhagic fevers, and the ELISA technique itself. This broad experience has already strengthened his ability to manage and direct the collection and forwarding of specimens for the use of USAMRIID, and will be invaluable as measures to speed the diagnosis of LF by means of ELISA become available.

During the summer of 1986 and prior to his coming to the United States Mr. Valley-Ogunro conducted plasmapheresis for LFIP at CLH and Phebe Hospital (PH). Since his return to Liberia he has continued this activity in almost monthly trips to the two hospitals. During his absence from Liberia a large backlog of serological specimens awaiting IFA testing accumulated; he is busy making up for his five months away from his laboratory at the LIBR.

Andrew K. Cole, M.D., has developed a rural health program in the town of Kolahun about 100 miles to the northwest of Zorzor, where CLH is located. Dr. Cole is awaiting the delivery of a solar-powered

freezer to investigate LF infections in surrounding villages where it is endemic (11,12). A goal of his during the remainder of the project will be to determine the incidence of LF in the region about Kolahun, an area which will be appropriate for field studies of a LV vaccine when one is prepared. He also hopes to develop techniques to aid primary health care workers in the diagnosis of LF.

Dr. Mark H. Monson, Director of CLH, continues to furnish invaluable help to the clinical aspects of the investigation; he will be responsible for actual patient management during the therapeutic trials, in addition to his clinical and administrative responsibilities at the Hospital.

The principal investigator, Dr. John D. Frame, visited Liberia in May, 1987. In the United States he coordinates project activities with the program at USAMRIID. In Liberia he reviews the LF program together with Dr. Aloysius Hanson, Director of the LIBR. He also reviews the work of laboratory personnel in the field stations, and discusses clinical aspects of LF with the medical and nursing staffs to help them define new goals for the investigation and management of LV infections. In May he participated in the USAMRIID sponsored Symposium, Hemostatic Impairment Associated with Hemorrhagic Fever Viruses, May 26-29, 1987.

Plasmapheresis

Plasmapheresis was interrupted by Mr. Valley-Ogunro's five months' absence in the United States, and by the breakdown of the refrigerated centrifuges, first at CLH and then at PH. When the centrifuges had been repaired the ultra-freezer at the LIBR broke down, and it did not seem wise to collect more units than could be stored in "borrowed" freezer space from other programs in the LIBR. In spite of these handicaps 153 LFIP units were obtained during the year; 83 have been forwarded to USAMRIID, or are awaiting shipment (Appendix, Table 1). It is expected that by the end of the autumn the total number of units obtained from the donors will reach our planned levels.

In order to replenish the number of plasma donors Mr. Valley-Ogunro finds it necessary on each trip to the field sites to spend at least one day traveling to the villages where former LF patients live. In order to enhance the likelihood that the plasma will have NA at the LNI of 0.3 acceptable to USAMRIID, he delays his search for donors for six months after the onset of illness. He is able to find the former patients because of careful notation of their place of residence at the time they are in the hospital; with the help of Mr. Boakai Kamara of the CLH Community Health program he is able to persuade most to accept plasmapheresis.

Lassa fever cases

The identification of cases of LF has been important in the elucidation of the significance of the disease in Liberia, and to find potential donors of LFIP. For the most part this activity was confined to CLH and PH during the year.

The diagnosis of LF is made by the serological testing of febrile patients and by viral isolation. During Mr. Valley-Ogunro's time at USAMRIID it was possible to attempt virus isolation of patient sera from CLH and PH to a greater extent than in some recent years.

Comparison of the results tabulated last year (1), when virus isolation was incomplete, with amended statistics presented now illustrates the significance of virus isolation in the diagnosis and determination of the frequency of LV infections. In the Annual Summary Report, July 31, 1986, it was noted the number of cases of LF and possible Lassa fever (see following paragraph) at CLH for ten months was 29, an incidence of 0.24 of febrile patients, and at PH, 10 for an incidence of 0.04. It was suggested that if both virus isolation and serodiagnosis had been attempted on all patients the number of cases would have been 33 and 14, respectively. When virus isolation was in fact attempted in all febrile patients during the year the revised number of cases was 33 and 16 for CLH and PH, respectively (Appendix, Table 2).

As noted previously (5) the diagnosis of LF depends upon the isolation of LV from the serum, or by seroconversion or a fourfold rise in antibody titers in paired specimens by means of the IFA technique. In cases in which a single specimen is obtained the diagnosis of "Possible LF" (PLF) is made if IFA titers are 1:64 or higher.

Between May and August, 1986, LV was isolated from six of 57 febrile patients at CLH, and seven of 83 at PH. Isolation from specimens obtained since September, 1986, has not yet been completed. Because of the absence of the Field Investigator from Liberia for much of the past year not all specimens have been tested to their end-point titers as yet. For these reasons an attempt to tabulate the incidence of LV in these hospitals since May, 1986, is not yet feasible.

In spite of the value of virus isolation in the determination of the incidence of LV in patients with fever, it is not anticipated that in the future virus isolation will be performed routinely in specimens of febrile patients. It appears that the time required for this purpose will be spent more usefully in other aspects of LF research. If ELISA proves reliable in field tests, the relatively rapid diagnosis of LF should be possible. Virus isolation will then be attempted only in those patients selected by ELISA for therapeutic trials.

Passive immunotherapy

Records are at hand for six patients who received plasma infusion at CLH between June and August, 1986; all survived. In two the diagnosis was confirmed by LV isolation; virus was not found in the sera of four, and titrations for LV antibody are not yet complete.

It is now standard policy at PH that infusion of LFIP must be considered whenever the clinical diagnosis of LF is made, and carried out if the history indicates that no more than 10 days have elapsed since the onset of illness. Between April, 1986 and February, 1987, LFIP was administered to nine patients. Lassa virus was isolated from two adult males; both recovered. Possible Lassa fever with a high virus antibody titer in one specimen was the diagnosis of one recipient, a pregnant female who died. Isolation of LV was unsuccessful in her and in three others, and was not attempted in another three, one of whom expired. Both patients who died received LFIP after the 10th day of illness; administration by the tenth day of illness is apparently a crucial factor in the use of LFIP (9). Serological tests have not been carried out to end points in all specimens.

Most LFIP units were administered "blind", that is, without the physician's knowledge of the LNI of the NA present. Therapeutic units of LFIP may be defined as the product of the LNI and the volume of the infusion in milliliters. The dose of therapeutic units received ranged from 200 to about 1100. The use of LFIG will likely permit a standard dose to be administered, a fact that will assure approximately equal potency of treatment for all patients.

Because of the small numbers of patients involved and the still unresolved issue of diagnosis in some, evaluation of the effectiveness of therapy does not appear possible at this time.

Conclusion

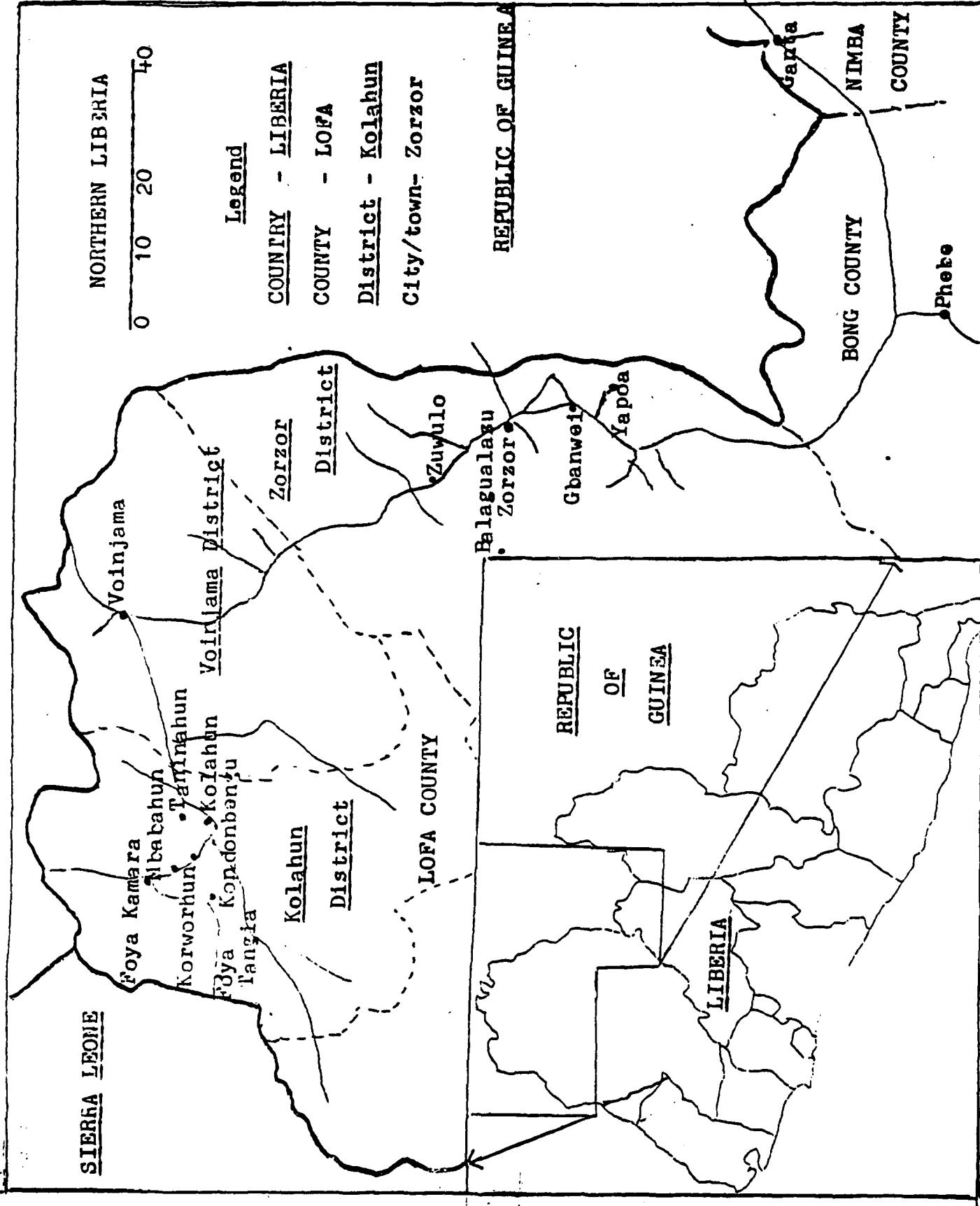
The instruction of the Field Investigator at USAMRIID in the techniques involved in the diagnosis of LF, and his personal relationships with the scientific and administrative staff there are considered to have strengthened the program of LF research in Liberia. A concomitant temporary slowing of the program of LFIP acquisition, and the delays in serological testing resulting from Mr. Valley-Ogunro's absence for five months will be compensated for early in the coming year.

Passive immunotherapy, as well as the trial of ribavirin among LF patients, using change in levels of viremia as the criterion for evaluation of results, await the more rapid and accurate diagnosis of LF which is likely to accompany the use of the ELISA test for viral antigen. It is expected that this technique will be field-tested, and it is hoped, come into regular use during the coming year.

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Appendix Tables.

Table 1. Lassa fever immune plasma units collected July 1986 - June 1987.

| <u>Donor</u> | <u>Date of illness</u> | <u>Date of donation</u> | <u>IFA titer*</u> | <u>LNI# Jos</u> | <u>Mac</u> | <u>Number of units Collected</u> | <u>USAMRIID**</u> |
|--------------|------------------------|-------------------------------|-------------------|-----------------|------------|----------------------------------|-------------------|
| DaBa | 11/82 | 5/12/86 3/27/87 4/29/87 | 16 | 1.6 | 2.2 | 2 2 | 2 |
| GoCo | 3/85 | 4/23/86 6/23/87 | 32 16 | 2.2 | 2.2 | 2 | |
| DaDo | 04/77 | 9/9/86 3/24/87 4/30/86 | | 0.5 | | 2 2 2 | 1 2 |
| MaFa | | 12/5/85 6/23/87 | - | 0.3 | 0.3 | 2 | |
| KeFl | 1983 | 9/11/86 3/24/87 4/30/87 | | 1.3 | | 2 2 2 | 1 2 2 |
| LoFl | 01/82 | 5/14/86 4/28/87 | 8 | 1.6 | 1.9 | 2 2 | |
| JoGa | 1976 | 9/10/86 3/25/87 | | 0.8 | | 2 2 | 1 2 |
| JoHo | 3/85 | 6/24/86 3/24/87 4/29/87 | 4 | | | 2 2 | 2 2 |
| DaJa | 10/84 | 9/11/86 3/24/87 | | 0.4 | | 2 2 | 1 |
| IrJo | 02/83 | 9/11/86 3/25/87 | | 2.2 | | 2 2 | 1 2 |
| BoKa | 10/82 | 9/11/86 3/27/87 4/28/87 | | 1.2 | | 2 2 2 | 1 2 |
| GKa | | 6/27/87 | - | | | 2 | |

Table 1 (cont)

| <u>Donor</u> | <u>Date of illness</u> | <u>Date of donation</u> | <u>IFA titer*</u> | <u>LNI#</u> | <u>Number of units Collected</u> | <u>USAMRIID**</u> |
|--------------|------------------------|---|-------------------|-------------|----------------------------------|-------------------|
| | | | | Jos Mac | | |
| JohKe | 3/84 | 9/10/86 4/28/87 | 8 4 | 2.1 | 2 2 | 2 2 |
| MuKe | 9/84 | 6/24/86 6/22/87 | - | 0.3 | 2 | |
| JoKo | | 4/24/86 6/22/87 | 4 8 | 0.3 0.4 | 2 | |
| GaKoII | 7/83 | 9/11/86 | | 0.3 | 2 | 2 |
| YaKo | 10/81 | 6/25/86 3/25/87 | 8 | 1.4 | 2 | 1 |
| DaKo | 10/81 | 9/11/86 3/25/87 4/28/87 | | 3.9+ | 2 2 2 | 1 2 2 |
| KeKo | 7/82 | 10/8/85 3/26/87 4/29/87 | 16 | 1.3 1.8 | 2 2 | 2 2 |
| EsKn | | 9/11/86 3/27/87 | | 0.4 | 2 2 | 2 |
| KoMa | | 9/9/86 3/27/87 4/30/87 | | 0.4 0.04 | 2 2 2 | 1 |
| KaMa | 03/83 | 9/10/86 3/25/87 | | 1.4 | 2 2 | 1 2 |
| KrMa | | 4/30/87 | | | 2 | 2 |
| NoMa | 11/82 | 6/25/86 4/29/87 | 16 | 3.1+ | 2 2 | 2 2 |
| JoMi | | 6/21/86 6/22/87 | 16 | | 2 | |
| JaMo | ? | 5/12/86 9/9/86 3/26/87 4/29/87 | 8 | 0.3 0.6 | 2 2 2 | 1 |

Table 1 (cont)

| <u>Donor</u> | <u>Date of illness</u> | <u>Date of donation</u> | <u>IFA titer*</u> | <u>LNI#</u> | <u>Number of units Collected</u> | <u>USAMRIID**</u> |
|--------------|------------------------|--|-------------------|----------------|----------------------------------|-------------------|
| ErRi | 6/84 | 6/25/86 4/28/87 | 128 | 1.7 | 2 | 2 |
| DaSu | 01/83 | 9/10/86 3/26/87 4/30/87 | | 0.5 | 2 2 2 | 1 2 1 |
| YaTa | 9/92 | 8/8/85 9/10/86 3/26/87 | 8 | 0.6 1.4 1.0 | 1 2 | 1 2 |
| JoTo | | 6/22/87 | 32 | | 2 | |
| BeTo | 2/84 | 6/25/86 3/27/87 4/30/87 | 128 | 1.8 | 2 2 | 2 2 |
| DaTo@ | 03/83 | 12/3/85 9/9/86 4/30/87 | 8 | 4.1+ | 2 2 | |
| NoTo | 1984 | 9/12/86 3/27/87 4/30/87 | | 2.2 | 2 2 2 | 1 2 2 |
| GeTu | | 6/23/87 | 64 | | 2 | |
| YaVa | 02/82 | 5/13/86 9/12/86 4/29/87 | 8 | 1.0 0.8 0.9 | 2 2 | 1 2 |
| BeVa | 8/78 | 3/19/86 9/9/86 4/28/87 | ? | 1.0 0.9 0.7 | 2 2 | 2 |
| JoVa@ | 1976 | 10/29/85 6/23/87 | ? | 0.6 0.6 | 2 | |
| KlVe | 07/83 | 6/25/86 3/25/87 | 32 | 0.7 | 2 | 2 |
| RaVe | 12/82 | 5/13/86 9/10/86 3/25/87 4/29/87 | ? | 0.4 0.2 1.1 | 2 2 2 | 2 2 2 |

Table 1 (conc)

| <u>Donor</u> | <u>Date of illness</u> | <u>Date of donation</u> | <u>IFA titer*</u> | <u>LNI#</u> | | <u>Number of units Collected</u> | <u>USAMRIID**</u> |
|--------------|------------------------|-------------------------------|-------------------|-------------|-----|----------------------------------|-------------------|
| MoWo | 04/77 | 9/10/86 3/24/87 | | 2.2 | | 2 2 | 1 1 |
| MaZa | 07/82 | 5/14/86 9/12/86 3/26/87 | 16 | 0.9 0.8 | 0.7 | 2 2 | 2 |
| | Total | | | | | 153 | 83 |

* Expressed as reciprocals of indirect fluorescent antibody titers. Tests performed at the LIBR. Reactivity of reagent varies from batch to batch, and comparisons of values must be made with caution. Not all tests completed at the time of preparation of the Annual Summary Report.

Log Neutralization Index; Josiah (Jos) and Macenta (Mac) strains of Lassa virus used as reagents. Tests performed at USAMRIID; not all completed at the time of the preparation of the Annual Report. If no tests have been performed to date on specimens submitted during the past year, the most recent previous determination is included, whenever possible showing LNI's to both strains.

** In general, specimens were forwarded to USAMRIID which showed an LNI of at least 0.3. Some specimens forwarded before LNI had been determined.

● Found to be HBsAg positive after LFIP units had been forwarded to USAMRIID.

Table 2. Patients with Lassa fever, Curran Lutheran Hospital (CLH) and Phebe Hospital (PH), July 1, 1985 - April 30, 1986, revised from Annual Summary Report, July 31, 1986

| Hospital | No tested | Lassa Fever Virus Isolation | Serocon- version | Total (Rate) | Possible LF (High IFA titers | Total, LF and possible pos. | Other IFA pos. <u>LF(Rate)</u> |
|---------------------|--------------|-----------------------------------|---------------------|-----------------|------------------------------------|-----------------------------------|---|
| CLH | | | | | | | |
| 7/1/85- 11/30/85 | 73 | 13 | 4 | 17 (0.23) | | 17 (0.23) | 5 |
| 12/1/85- 4/30/86 | 47 | 7 | 4 | 11 (0.23) | 5 | 16 (0.34) | 1 |
| Total, CLH | 120 | 20 | 8 | 28 (0.23) | 5 | 33 (0.28) | 6 |
| PH | | | | | | | |
| 7/1/85- 5/15/86 | 221* | 7* | 7 | 14 (0.06) | 2 | 16 (0.07) | 16 |

* Virus isolation not attempted in 96 specimens of this series.

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